Import and processing of genetically modified maize MIR162

COGEM advice CGM/101019-04

The present application by Syngenta Crop Protection AG (file EFSA/GMO/DE/2010/82) concerns import and processing for use in feed and food of the genetically modified maize line MIR162. Cultivation is not part of this application.

Maize line MIR162 expresses the vip3Aa20 gene conferring resistance to certain lepidopteran insects. In addition, this maize line expresses the pmi gene which acts as a selectable marker enabling transformed plant cells to utilize mannose as a carbon source.

Previously, COGEM issued positive advices on import and processing of maize lines Bt11xMIR162xGA21 and Bt11xMIR162xMIR604xGA21. Maize line MIR162 was evaluated in these applications. In the current application the bioinformatic analysis of the junctions between the insert and the maize genomic DNA was updated. The molecular characterization of maize line MIR162 meets the criteria of COGEM.

During its long domestication process, maize has lost its ability to survive in the wild. In the Netherlands, the appearance of maize volunteers is rare and establishment of volunteers in the wild has never been reported. There are no reasons to assume that the introduced traits or interactions between the transgenic proteins will increase the potential of maize to establish feral populations. The introduced genes cannot spread to closely related species since wild relatives of maize are not present in Europe.

In view of the above, COGEM is of the opinion that the risks for humans and the environment associated with import and processing of maize line MIR162 are negligible. A food/feed safety assessment is carried out by other organizations. Therefore, COGEM abstained from advice on the potential risks of incidental consumption.

Introduction

The scope of the present notification (EFSA/GMO/DE/2010/82) by Syngenta Crop Protection AG concerns import and processing of maize line MIR162. This maize line has recently been authorized for cultivation, import and processing in the United States of America (2010), Canada (2010) and Brazil (2009).[1] Maize line MIR162 contains the *vip3Aa20* and *pmi* genes which are constitutively expressed. As a result, the maize line is resistant to certain lepidopteran insects and able to use mannose as a carbon source.

Previous COGEM advices

COGEM has previously issued positive advices on import and processing of maize lines Bt11xMIR162xGA21 and Bt11xMIR162xMIR604xGA21.[2,3] Maize line MIR162 is a parental line of these two maize lines.

Aspects of the crop

Maize (*Zea mays* L.) is a member of the grass family *Poaceae*. Maize is cultivated as an agricultural crop, originating from Central America. Although insect pollination cannot be completely excluded, maize is predominantly wind pollinated.[4,5] According to literature, pollen viability varies between 30 minutes and 9 days.[5,6,7] In Europe, no wild relatives of maize are present and, therefore, hybridization with other species cannot occur.[13]

Throughout the world the appearance of volunteers is very rare. Seed kernels are the only survival structures of maize.[13] Due to the structure of the corn cob (ear on a stiff central cob enclosed in husks) natural dissemination of the kernels rarely occurs. Maize needs human intervention to disseminate its seed.[13] In addition, kernels exhibit poor dormancy resulting in a short persistence. Besides, maize can only survive within a narrow range of climatic conditions and, originally a subtropical crop, maize is naturally frost-sensitive.[8] Maize is very sensitive to weed competition.[14] During the long process of domestication, maize has lost the ability to survive in the wild.[13] Establishment of maize plants in the wild has never been observed in the Netherlands and COGEM is not aware of any reports of wild maize plants elsewhere in Europe.

Molecular characterization

Maize line MIR162 was produced by *Agrobacterium tumefaciens* mediated transformation. As a result MIR162 contains the following gene cassettes.

Maize line MIR162

The *vip3Aa19* and *pmi* gene cassettes were introduced in maize line MIR162 via *A. tumefaciens* mediated transformation.

The *vip3Aa19* gene cassette consists of the following elements:

- ZmUbiInt promoter, derived from the *Z. mays* polyubiquitin gene; provides constitutive expression in monocots
- *vip3Aa19* gene, modified version of the native *vip3Aa1* gene from *Bacillus thuringiensis* strain AB88
- iPEPC9 intron, intron #9 from the phosphoenolpyruvate carboxylase gene from *Z. mays*
- 35S terminator, derived from CaMV

The *pmi* gene cassette consists of the following elements:

- ZmUbiInt promotor, derived from the *Z. mays* polyubiquitin gene; provides constitutive expression in monocots
- *pmi* gene, from *Escherichia coli*; catalyzes the isomerization of mannose-6-phosphate to fructose-6-phosphate
- NOS, terminator sequence from the nopaline synthase (*nos*) gene of *A. tumefaciens*

Molecular analysis

Maize line MIR162 was previously assessed by COGEM in light of the applications for import and processing of maize Bt11xMIR162xGA21 and Bt11xMIR162xMIR604xGA21.[2,3] The molecular analysis of maize MIR162 is described below.

By Southern blot hybridization with a probe spanning the entire backbone region of the vector the applicant showed that the vector backbone is not present in maize MIR162. In addition, Southern blot hybridizations with a *vip3Aa19-, pmi-*, ZmUbiInt promoter- and with a NOS terminator-specific probe showed that one copy of the *vip3Aa19* gene, *pmi* gene and NOS terminator and two copies of the ZmUbiInt promoter were present. These results are expected when one copy of the insertion cassette is present in maize MIR162.

Sequence analysis of the insert confirmed that the insertion cassette is intact, but indicated that the right border (RB) and the left border (LB) ends of the insert are truncated compared to the vector. In MIR162 the entire RB along with two non-coding base pairs is missing, and the entire LB is missing along with 32 non-coding base pairs. Sequence analysis indicated that in MIR162 two nucleotides of the *vip3Aa19* gene had changed. One of the nucleotide changes leads to a different amino acid, whereas the other nucleotide change does not influence the amino acid sequence. To indicate the difference with the *vip3Aa19* gene the applicant designated the *vip* gene that is present in MIR162 *vip3Aa20.*

The applicant also sequenced 1000 base pairs of the 5' and 3' flanking regions and showed by BLAST analysis that these regions are maize genomic DNA. Bioinformatic analysis of the 3' flanking region showed that this region is homologous to maize genomic DNA. Bioinformatic analysis of the 5' flanking region indicated that part of this region is significantly homologous to *Dissociation1* (*Ds1*) related transposable elements. The region of homology is located more than 500 base pairs from the insert. A *Ds1* transposable element may transpose if an *Activator* (*Ac*) element is present. If the *Ds1* element would transpose, part of the maize genomic DNA that flanks the *Ds1* element may be deleted. In maize, deletions caused by *Ds1* transposable elements rarely extend into the host genome.[9] The largest amount of host DNA reported to be deleted was 36 base pairs.[10] Since the *Ds1* transposable element is located at 500 base pairs from the insert, it is unlikely that the insert would be affected. Moreover, transposable elements are naturally occurring elements. Therefore, any effect of the *Ds1* transposable element is regarded as baseline.

Bioinformatic analysis of the junctions of the insert and the maize genomic DNA identified twelve nucleotide sequences that were delimited by putative stop codons. For the previous applications concerning import and processing of maize lines Bt11xMIR162xGA21 and Bt11xMIR162xMIR604xGA21 these sequences were analyzed for their homology to toxins or allergens. In March 2010 the sequences were reanalyzed. None of the nucleotide sequences were homologous to toxins or allergens.

Properties of the introduced genes conferring insect resistance

Maize line MIR162 contains the *vip3Aa20* gene, which was derived from *B. thuringiensis.* The *vip3Aa20* gene is a modified version of the *vip3Aa1* gene which encodes a vegetative insecticidal protein (VIP3Aa20). The mode of action of Vip3A proteins is similar to that of the well-known δ-endotoxins. Like δ-endotoxins, Vip3A is processed in the insect gut. The resulting fragment binds to epithelial cells of the midgut of susceptible insects which results in the formation of pores in the membranes of the gut cells of the insect and finally in the insect's death. [11,12]

Insects that are susceptible to the Vip3Aa20 protein are e.g. the corn earworm (*Heliothis zea*), the black cutworm (*Agrotis ipsilon*), the fall armyworm (*Spodoptera frugiperda*) and the Western bean cutworm (*Striacosta albicosta*).

Properties of the introduced selection marker

MIR162 also contains the *pmi* (*manA*) gene, which encodes the phosphomannose isomerase (PMI) enzyme. As a result maize plants are able to use mannose as a carbon source. Mannose is phosphorylated to mannose-6-phosphate (M6P) which can be converted to fructose-6-phosphate with the help of PMI. In non-transgenic maize plants conversion of M6P will not occur. M6P will accumulate, block glycolysis, and inhibit plant growth.

The ability to use mannose as a carbon source is used to select transformed cells in cell cultures.

Environmental risk assessment

During the long process of domestication, maize has lost the ability to survive in the wild.[13] Maize needs human intervention to disseminate its seed.[13] Maize kernels exhibit poor dormancy resulting in a short persistence. Maize is very sensitive to weed competition and cannot persist as a weed.[13,14] Furthermore, maize is naturally frost sensitive and can only survive within a narrow range of climatic conditions. In the Netherlands, volunteers are rarely found and establishment of maize plants in the wild has never been observed. In Europe, no wild relatives of maize are present and, therefore, hybridization with other species cannot occur.[13]

Maize MIR162 expresses the *vip3Aa20* and *pmi* genes. As a result, the maize line is resistant to certain lepidopteran insects and able to use mannose as a carbon source. The current application concerns import and processing. In case of spillage maize kernels may be released into the environment. Maize kernels can only survive within a narrow range of climatic conditions. The introduced traits do not increase the ability of maize kernels to survive in the environment. In addition, the applicant carried out an agronomic assessment for MIR162. This assessment does not give any indication to assume that MIR162 has an increased survivability compared to conventional maize lines. In view of the above, there are no reasons to assume that maize MIR162 has an increased potential for the establishment of feral populations in case of incidental spillage.

Since 2008, COGEM abstains from advices on the potential risks of incidental consumption in case a food/feed safety assessment is already carried out by other organizations. This application is submitted under Regulation (EC) 1829/2003, therefore a food/feed safety assessment is carried out by EFSA. Other organizations who advice the competent authorities can perform an additional assessment on food safety although this is not obligatory. In the Netherlands a food and/or feed safety assessment for Regulation (EC) 1829/2003 applications is carried out by RIKILT. Regarding the risks for food and feed, the outcome of the assessment by other organizations (EFSA, RIKILT) was not known at the moment of the completion of this advice.

General surveillance

General surveillance has been introduced to be able to observe unexpected adverse effects of genetically modified crops on the environment. The setting or population in which these effects might occur is either not, or hardly predictable. Usually, in case of import and processing of genetically modified maize general surveillance is supposedly carried out by operators involved in the handling and use of viable maize.

Recently, COGEM formulated criteria on which general surveillance plans concerning Dutch applications for import and cultivation of gm-crops have to comply.[15] COGEM concluded that the general surveillance plans could be improved by a guarantee that operators will monitor for unanticipated effects. In the present general surveillance plan on MIR162 maize the authorization holder states that the operators have agreed to provide information relevant to the monitoring of MIR162 maize to the authorization holder. More important, it is stated that the authorization holder will be able to give evidence that the operators collect this information.
COGEM is content that her recommendation for improvement of the general surveillance plan has been followed and considers the general surveillance plan sufficient for import and processing of MIR162 maize.

Advice

COGEM has been asked to advice on import and processing of maize line MIR162. The molecular characterization of maize line MIR162 meets the criteria of COGEM.

Maize has lost the ability to survive in the wild. In addition, maize needs human intervention to disseminate its seed. In the Netherlands, volunteers are rare and establishment of maize plants in the wild has never been observed. There is no reason to assume that expression of the *vip3Aa20* and *pmi* genes increase the potential of maize to establish feral populations in case of incidental spillage. In addition, an agronomic assessment of MIR162 did not give any indication to assume that MIR162 has an increased survivability compared to conventional maize lines. Introgression of the introduced genes into closely related species cannot occur, as wild relatives of maize are not present in Europe.

In view of the above, COGEM is of the opinion that the risks for humans and the environment associated with import and processing of maize line MIR162 are negligible. A food/feed safety assessment is carried out by other organizations. Therefore, COGEM abstained from advice on the potential risks of incidental consumption.

Additional remark

MIR162 expresses the *vip3Aa20* gene. This gene is a modified version of the *vip3Aa19* gene which is a codon optimized version of the *vip3Aa1* gene. Some, but limited, information on the insect specificity of the Vip3Aa1 and the Vip3Aa19 proteins is available. However, this information cannot be directly extrapolated to the Vip3Aa20 protein since the amino acid sequence of Vip3Aa20 is not completely identical to the Vip3Aa19 or the Vip3Aa1 protein.

In case of import and processing the exposure of non-target organisms is limited to unintended release of MIR162 maize by spillage. Therefore, COGEM is of the opinion that in the current application, the lack of data on the specificity of the Vip3Aa20 protein is not a reason for concern. However, in case of cultivation non-target organisms will be exposed to Vip3Aa20. For such an application COGEM considers data on the specificity of the Vip3Aa20 protein essential.

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